United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

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Title: Microbiological Media		
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1. Purpose:

To provide standard procedures to ensure that all media and reagents used in Microbiological Data Program (MDP) laboratories meet analytical standards for promoting good growth and retention of microorganisms.

2. Scope:

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program. All of the procedures and measures required under this SOP must be documented. Records must be kept in laboratory logbooks.

3. Outline of Procedure:

5.1 Receipt of Dehydrated Media, Reagents, and Ingredients
5.2 Storage of Dehydrated Media, Reagents, and Ingredients
5.3 Productivity/Selectivity Testing of Media and Reagents
5.4 Visual Examination of Media
5.5 Reagents
5.6 Salmonella Antisera
5.7 Records
5.8 Results
5.9 Limitations

4. References:

- AOAC International, Quality Assurance Short Course, Quality Assurance for Microbiological Laboratories, 481 North Frederick Ave., Suite 500 Gaithersburg, MD
- BBL Quality Control and Product Information Manual for Plated Media, January 1996

- Compendium Of Methods for the Microbiological Examination of Foods, Third Edition, 1992, American Public Health Association, Washington, D.C.
- Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, U.S. Department of Health and Human Services, Public Health Service, May 1993
- Becton Dickinson and Company/Difco Laboratories, Difco Manual, 11th Edition, 1998
- Standard Methods for The Examination Of Dairy Products, 16th Edition, 1992, American Public Health Association, Washington, D.C.
- USDA, FSIS Microbiology Laboratory Guidebook, 3rd Edition, 1998, Washington, D.C.
- Quality Control in Microbiology, Department of Health and Human Services, Public Health Service, by J. Michael Miller, May 1993

5. Specific Procedures:

5.1 Receipt of Dehydrated Media, Reagents, and Ingredients

a. Containers of media and reagents shall be dated upon receipt. An inventory control manual shall contain the following information for each shipment:

1. Manufacturer

2. Quantity Received, i.e., size and number of containers

3. Date Received

4. Date Opened

5. Location where medium/reagent is to be stored

6. Initials of persons receiving and placing each item into stock

b. Each lot of medium/reagent shall be inspected upon receipt or upon opening for volume, tightness of closure, clarity, color, consistency, and completeness

of label. This information must be documented in the laboratory quality assurance (QA) logbook.

c. All media and reagent bottles shall be labeled with the date received and the date opened and the bottles shall be initialed.

5.2 Storage of Dehydrated Media, Reagents, and Ingredients

a. Follow the manufacturer directions for handling and storage of media/reagents. The following are general guidelines:

1. Store dehydrated media in tightly capped bottles in a cool, dry place protected from light. If specified, keep under refrigeration and in the dark.

2. Keep no more than 6-12 month's supply on hand. Use older stock first. Do not exceed the supplier's expiration date.

3. When a bottle of media/reagent is placed into service, it shall be logged out of a media/reagent inventory book. The date and initials of the person logging out the media shall appear in this logbook. If a laboratory has an alternative means of inventory control that satisfies this requirement, MDP will consider it as acceptable.

4. Dehydrated media and reagents should be free-flowing powders or crystals. Media containing dyes shall be protected from light by storage in a dark room or a dark glass bottle or by wrapping the container with foil.

b. Media shall be stored in alphabetical order.

c. The initials of the person opening and the date media/reagent is opened shall appear on the container. Also, the date of receipt is to be written on the

container. This information shall be transferred from outside boxes or covers to containers when opened.

5.3 Productivity/Selectivity Testing of Prepared Media and Reagents

a. Organisms

1. Pure, fresh (18 to 24 h) cultures of reference strains commercially available or well-characterized isolates shall be used.

2. Two or more organisms are needed to check the growth characteristics, selectivity, or biochemical reactivity of a medium. Use appropriate references to determine the organisms to be used.

b. Maintenance of Reference Organisms

1. The laboratory shall maintain three levels of cultures for use:

a. a working set of cultures maintained on tryptic soy agar (TSA) or TSA/yeast extract (TSA/YE).

b. a backup set of cultures maintained on TSA or TSA/YE slants.

c. a stock culture maintained on beads or in button form and frozen at -70^0C or some other long-term storage mechanism. If desired, the laboratory may use other appropriate medium specific for certain organisms, but it is recommended that extremely rich media not be used for stock culture maintenance.

2. Cultures from slants shall not be transferred more than five times. Retrieve a new bead or revive a stock culture from long-term storage to start a fresh culture every 3 to 4 months.

c. Preparation of Reference Organisms for Testing

1. Use fresh cultures (18-24 hours old) for testing media.

2. For selective media requiring tests for ability to inhibit organisms or when determining the ability to support growth, a dilute inoculum may be needed. The dilute suspension will give greater assurance that the medium will support growth of a small number of organisms or inhibit the reference organism.

3. Prepare a standardized inoculum to a turbidity equivalent of a 0.5 McFarland standard and use a 3-millimeter loop to deliver a known amount to test the media.

d. Acceptable Performance of Solid Selective Media

1. A selective medium is one that contains a substance that suppresses the growth of specific groups of microorganisms.

2. The following testing shall be performed on one bottle of each manufacturers lot of selective media. If a certificate is obtained from the manufacturer stating that the media was evaluated under National Committee for Clinical Laboratory Standards requirements, the following testing does not need to be performed.

3. The laboratory shall test for efficiency of selective plating media as follows: Each new lot of selective plating medium must be tested for its efficiency in growing organisms such as *Salmonellae*, which as a group, has a diverse spectrum of serotypes. To test media efficiency for *Salmonella*, dilute 18-h broth cultures of *Salmonella typhimurium*, 10^{-5}, 10^{-6}, and 10^{-7} in Butterfield's phosphate diluent. For each dilution the laboratory shall use three plates of test medium (selective media) and three plates of trypticase soy agar. Each plate is to be

inoculated with 0.1 mL of culture dilution and the inoculum spread with sterile spreading rods. The plating shall be completed in 15 minutes after a dilution is made. The dilutions suggested here for *Salmonella*, usually result in a least one countable set of plates. If they do not, repeat the test on a new 18 h culture. The plates must be incubated at 35^0C for 48 h, and then the colonies counted.

$$\textbf{Percent (\%) efficiency} = \frac{\textbf{No. of colonies on plating medium}}{\textbf{No. of colonies on trypticase soy agar}} \times 100$$

The laboratory must test all *Salmonella* selective plating media in this manner. An efficiency of 75% as compared to trypticase soy agar with *S. typhimurium* is satisfactory. Colonies of most *Salmonellae* will appear to be larger and more numerous on the non-selective medium.

4. Testing of selective media for other species of microorganisms shall be performed on one bottle of each manufacturer's lot of selective media in a manner analogous to the *Salmonella* test.

e. Performance and Sterility Testing of Prepared Media Batches

A batch refers to all tubes, plates, or containers of media prepared at the same time and sterilized in the same autoclave using the same conditions. Laboratories that purchase prepared media are required to maintain back-up supplies of media and reagents. In cases of emergency, where media is prepared and used on the same day, all quality, performance, and sterility testing shall be performed concurrently with sample analyses. If the media proves faulty, the sample analysis must be discarded.

1. Each batch of media and reagents used in the MDP, whether prepared in the laboratory from dehydrated ingredients or purchased in prepared form shall be checked for performance and sterility.

2. Perform a performance test on each batch of selective, differential, and nutritive media using performance data supplied by the manufacturer or CDC as a guide for each organism. Recommended test parameters can be found in "Quality Control in Microbiology," by J. Michael Miller 1987 (This book has already been supplied to each laboratory participating in the MDP). The performance tests shall include sterility, ability to support growth, selective and/or inhibitory growth characteristics, and the biochemical reactivity of media in response to an appropriate culture.

3. To determine sterility and performance on small batches of media (less than 100 units) incubate, at a minimum, one uninoculated, one negative and one positive unit at the usual temperature for the same period of time as required in the specific analyses.

4. For larger batches of media, test 2% of the units. Depending on the batch size and the number of units to be tested, the laboratory may use any number of units desired for determining sterility and performance testing. The laboratory shall document the number of units incubated for uninoculated, positive, and negative controls. Based on the type of media being tested, the units tested shall be incubated at the usual temperature for the same period of time as required in the specific analyses.

5. Discard and autoclave units after incubation. Do not reuse sterility test media.

5.4 Visual Examination of Prepared Media

Examine prepared batches of media/reagents macroscopically for appropriate color, color changes, consistency, and dehydration. If a change is noted immediately in physical properties or if changes are noted over time discard the batch of media.

5.5 Reagents

a. The laboratory shall document the preparation of all reagents in QA logbooks. This includes all weights, calculations, and concentrations.

b. Sterile reagents are to be checked for sterility. Items such as sterile dilution blanks of phosphate buffer or saline are to be stored in an enclosed cabinet. They will be segregated and stored by date and batch number. From each batch of sterile dilution blanks of phosphate buffer or saline, a 1-mL aliquot from an uninoculated blank of the diluent will accompany the sample or group of samples in the appropriate medium.

c. All reagents shall be labeled with the name of the reagent and date prepared. Formulations and storage requirements will be recorded and maintained in the QA files and the laboratory QA logbook.

5.6 *Salmonella* Antisera

a. Each lot of *Salmonella* antisera shall be checked by use of positive and negative control antigens.

b. Antisera are to be kept refrigerated. Follow manufacturers recommendation for preparing antisera.

c. Surplus diluted antisera will be discarded on the day it is prepared.

5.7 Records

a. Records of media performance must be maintained for each batch of media tested.

b. Records shall include the lot number of media used and expiration date, the date media was prepared, organisms used, expected reactions, obtained reactions, results of sterility tests and visual examinations, problems, and

corrective actions. This information shall include the initials of the analyst performing the observations.

5.8 Results

a. Record results obtained on the quality control log and compare them with the expected results for each organism and media described.

b. If results are unacceptable, repeat the test. If the repeat test is unacceptable, discard the batch and document all problems and corrective actions taken.

c. Record results of visual examinations and discard unacceptable media.

5.9 Limitations

Media may fail quality control performance tests because the media was not prepared correctly or tests were not performed correctly.

a. The quality of prepared media depends on:

1. Accurately weighing the medium.

2. Carefully measuring the water.

3. Using water that is free of chlorine, copper, lead, and detergents and that has been determined to have suitable conductivity and ion content.

4. Using clean glassware.

5. Obtaining a homogeneous mixture before dispensing.

6. Proper pH adjustment.

7. Correct sterilization at the prescribed temperature, pressure, and time.

8. Using quality dehydrated media that has been properly stored.

b. Accurate quality control test results depend on:

1. Using fresh, pure, typical reference cultures.

2. Using a light inoculum for the testing of plating media and media tested for ability to inhibit growth.

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- Established standard procedures for quality assurance of microbiological media